The Interconnectedness of All Things

Approximately ten years ago, Sheue-Yann Cheng, Ph.D., Head of the Gene Regulation Section in CCR's Laboratory of Molecular Biology, teamed with an unusual lab partner—a mutant mouse. She made this mouse to study a rare inherited disease, resistance to thyroid hormone (RTH), caused by a mutation in one of two thyroid hormone receptor genes. RTH had been recognized for many years as a paradoxical deficit in thyroid hormone signaling that is seen despite elevated levels of thyroid hormone itself. But the disorder was only relatively recently traced to a receptor mutation that prevents hormone binding and the resulting transcriptional regulation. The mice she used to study the effects of this mutation turned out to be an important window into multiple physiological systems, including cancer.

Normally, laboratory mice live quite happily for 18 to 24 months, and yet Cheng's mice were starting to die at six months. "Our mice were dying!" exclaimed Cheng, "so we did autopsies." The Cheng team discovered that these mice had massively enlarged thyroid carcinomas.

Cheng immediately jumped on the opportunity to study what proved to be a model of follicular thyroid cancer. Thyroid cancers are one of the few cancers with a rising incidence around the world, particularly in women. Follicular thyroid cancer accounts for about 15 percent of the total thyroid cancer disease burden but has a poorer prognosis as compared to the dominant papillary variety. "Cancer is such a devastating disease. I felt that since I had been in the thyroid hormone field for so long, perhaps I could make a unique contribution."

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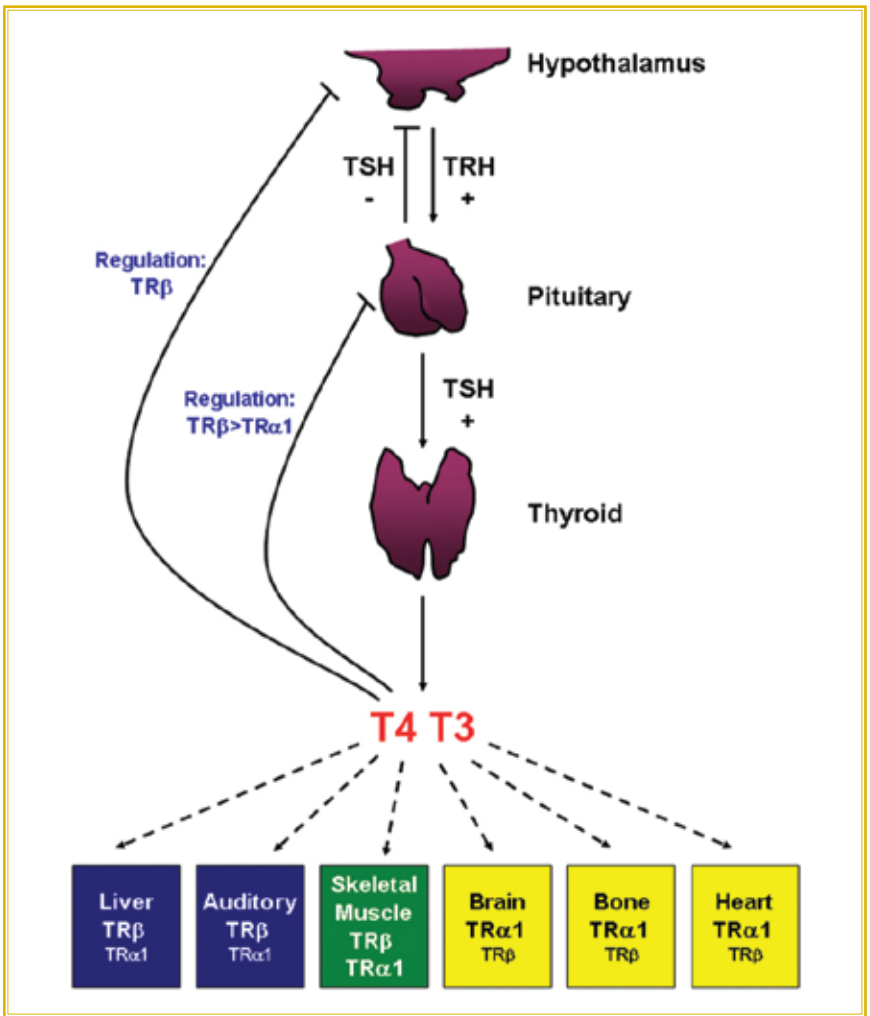
Spontaneous Tumor Generation

Thyroid hormones operate in a tightly controlled feedback loop involving the hypothalamus, pituitary, and thyroid glands. Activation of normal thyroid hormone receptors encoded by one of two genes—*THRA* or *THRB*—results in the downregulation of thyroid-stimulating hormone (TSH). TSH, as its name implies, encourages the growth and activation of cells in the thyroid gland.

The PV mutation that Cheng studies was derived from an RTH patient and encodes a mutation that shifts the translation of DNA by a single base pair near one end of *THRB*. This frameshift mutation results in a complete loss of binding of thyroid hormone receptor β to the thyroid hormone T3. In addition, the PV mutation acts in a dominant negative fashion, suppressing the function of the remaining normal TRβ receptor. Mice bearing this mutation, like people with RTH, have growth retardation and other hallmarks of reduced thyroid hormone signaling. RTH patients typically only have one mutated copy of the *THRB* gene; there has only been one report of a patient who had mutations in both copies. But homozygous mice bearing two copies of the PV mutation develop thyroid cancer.



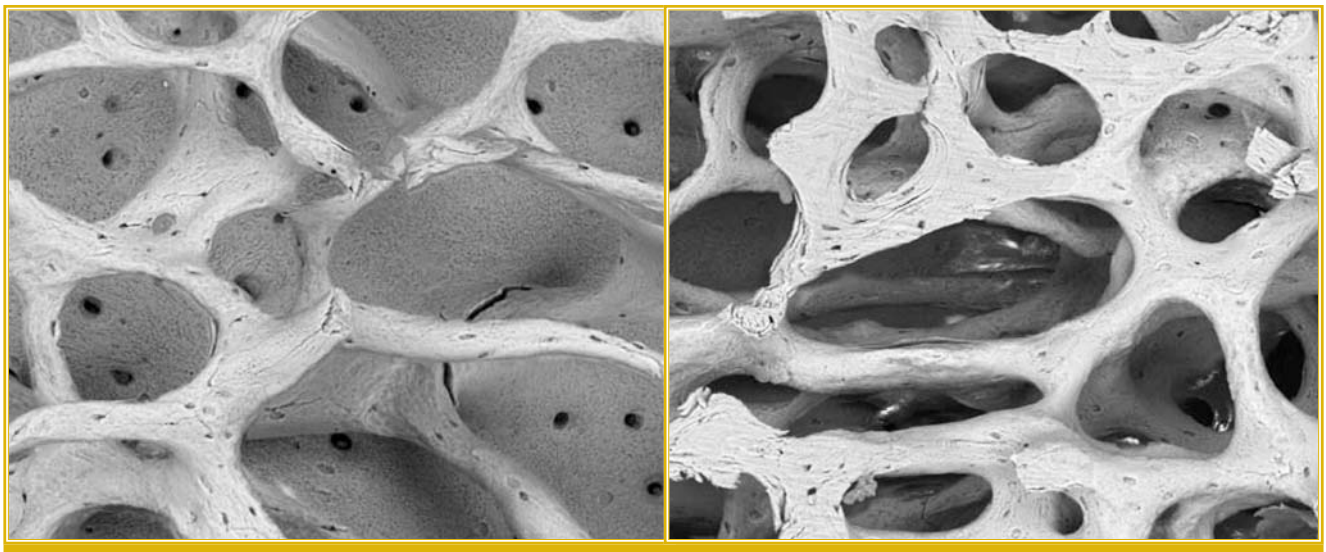
The hypothalamic-pituitary-thyroid (HPT) axis consists of a complex signaling network that regulates multiple organ systems. TSH = thyroid-stimulating hormone; TRH = thyrotropin-releasing hormone; T3, T4 = thyroid hormones; TR = thyroid hormone receptor. Image adapted from O'Shea et al., *Nuclear Receptor Signaling* (2006) 4, e011.

"When we developed this mouse model, there wasn't any other spontaneous mouse model of metastatic thyroid cancer," said Cheng. "As they got older, they just developed cancers. Eventually 100 percent of these mice develop thyroid cancer."

Several studies later, Cheng and her colleagues have characterized the progression and molecular changes associated with their mouse model of thyroid cancer. The cellular progression of the disease resembles the human situation. "We have studied so many of these mice," said Celine Guigon, Ph.D., a Postdoctoral Fellow in Cheng's laboratory. "And they all develop goiter around two months of age and then go on to develop cancer." Remarkably, even metastatic progression is observed reliably in these animals. "This mouse has a similar frequency of metastases to that seen in humans—around 25 to 30 percent for follicular carcinoma," said Cheng.

They have also found very strong correlations between the additional mutations observed in human follicular thyroid carcinoma and those seen in their PV mice. For example, they have established that the tumor suppressor PPARγ has reduced expression and activity in their mice and, intriguingly, that administration of a PPARγ agonist, rosiglitazone, blocked the development of metastasis.

Having validated their model as recapitulating many of the hallmarks of follicular thyroid carcinoma, Cheng and her colleagues hope to use the power of mouse genetics and molecular biology to gain novel insights into the disease. For example, their work has already shed light on the controversial role of TSH in these cancers. "Some patients who have elevated TSH have a high incidence of thyroid cancer; however, some patients with aggressive cancer express lower levels of TSH receptor," explained Cheng.



Scanning electron micrographs (SEM) of trabecular bone architecture in normal mice *(left panel)* and mice with mutations in the thyroid hormone receptor TRα. Image adapted from Bassett et al., *Scanning*, (Author manuscript; available in PMC 2009 July 6).

Cheng's team has found that in mice that are homozygous for the PV mutation, TSH levels are elevated about 200 times above levels in mice with only a single copy of the mutation. "TSH stimulates the proliferation of thyrocytes," said Cheng. "Together with the thyroid hormone receptor β mutation, these two altered signals stimulate cancer." Cheng and her colleagues have shown that elevated levels of TSH alone do not cause cancer, nor does the dominant negative action of the PV receptor mutation alone. Both are required. "As you know, cancer is a multigenetic disease," said Cheng. Her goal is to dissect the multiple molecular interactions critical to thyroid cancer formation and progression and bring the findings back to the clinic.

Fat and Bones

"Why do we need two thyroid hormone receptor genes?" asked Cheng. *THRA* and *THRB* encode TRα and TRβ receptors, respectively. TRα and TRβ are known to have different distributions in the body, and mice lacking the genes for each of these receptor subtypes have distinct functional deficits. But no case of RTH had ever been reported to be the result of a *THRA* mutation. "So we decided to target the PV mutation to the *THRA* gene and see what happens in the mouse," explained Cheng.

The lab is now studying lipid metabolism in these mice conferred by either *THRA* or *THRB* mutations; they are finding differences in regulation of both white adipocytes and lipid content of the liver. "We are focusing on lipid metabolism not only because our mice have distinct phenotypes, but because it is very important to know how thyroid hormone regulates lipid metabolism," said Cheng. Drug companies are interested in developing thyroid hormone analogs to accomplish therapeutic goals like lowering cholesterol. However, because of its pleiotropic actions on different receptor subtypes, a simple thyroid hormone analog would have too many side effects. "So there is a drive to devise analogs that are TR-subtype specific." Cheng hopes that her work will shed light on the potential effects of such specific analogs.

Meanwhile, Cheng has several collaborators who are using her mice to study diverse topics. "I cannot study everything in these mice," said Cheng. "They have a phenotype of interest to lots of investigators."

Graham Williams, Ph.D., Professor of Endocrinology at Imperial College in London, has worked with Cheng for several years.

"We had identified TRα as the major thyroid hormone receptor expressed in bone, and I developed an interest in *in vivo* models to investigate the molecular and physiological mechanisms of thyroid hormone action in bone," said Williams. "After seeing the early phenotype descriptions of the PV mutants, I was sure they would be very informative to our understanding of the skeleton. So, at one of the American Thyroid Association meetings, I introduced myself to Dr. Cheng and suggested that we work together on analyzing the skeletal phenotypes."

The differences in skeletal development between the two mice are marked—mice with PV mutations in the α receptor have retarded bone development characteristic of thyroid hormone deficit (hypothyroidism) whereas those with PV mutations in the β receptor develop an osteoporosis that is characteristic of elevated thyroid hormone (thyrotoxicosis). By comparing these mice, the investigators are able to tease apart the molecular mechanisms that regulate the skeletal response to thyroid hormone.

"Dr. Cheng and I have discussed and designed experiments, and members of her laboratory have provided bone samples from appropriate groups of mice to our laboratory so that we can perform the skeletal analyses," said Williams, describing the closeness of their collaboration.

In addition, their work together has resulted in some unique training opportunities. "One of my graduate students, Patrick O'Shea, was able to spend two years as a postdoctoral fellow in Dr. Cheng's lab at the NCI before returning to my lab in London for the third year through a European Union fellowship obtained as a direct result of our collaborations. His work with Dr. Cheng complemented his prior pharmacology background to enable him to secure an independent scientific career."

Gender Matters

When not in the operating theater, Electron Kebebew, M.D., Head of the Endocrine Oncology Section in CCR's Surgery Branch, has been trying to understand why the incidence of thyroid cancer is about four times as great in women as in men. He also wants to know why, paradoxically, men tend to have a more aggressive disease—typically presenting with metastases in the lymph node or lung at diagnosis—with higher rates of recurrence and reduced overall survival. "It is clinical and epidemiological data that no one understands," said Kebebew.

Given the gender disparity, some investigators have suggested that sex hormones may play a role in thyroid cancer. Studies in cell culture models have shown that estrogen administration leads to higher rates of growth in thyroid cancer cells. Kebebew's group has also done genomic studies in human thyroid cancer samples to demonstrate gene expression differences in papillary thyroid cancer in women as compared to men. Kebebew is collaborating with Cheng to see if they can study gender differences in her mouse models.

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Guigon, Cheng, and their colleagues have "improved" upon their original model of follicular thyroid cancer by combining the homozygous PV mutation with a mutation in one copy of the *PTEN* gene. *PTEN* mutations are also observed in human follicular thyroid carcinomas and the addition of this mutation to the PV mutations appears to accelerate the formation and progression of tumors. In addition, there is a gender difference in the manifestation of thyroid cancer in these mice, for example in the rate of metastasis.

"In conjunction with some *in vitro* experiments, we're using Cheng's model to see if sex hormones influence the rate at which the mice get follicular thyroid cancers, as well as their aggressiveness," said Kebebew. In order to study these questions, Kebebew's group is taking the mice at an early age and replacing the ovaries or testes with estrogen or testosterone implants, respectively. Half the mice receive sham implants without sex hormones so that the researchers also can compare the development of cancer with and without sex hormone. They will also study the gene expression differences in the mouse tumors and compare them with profiles of human disease.

"Other transgenic mice exist that develop thyroid cancer, but in none of those studies have people noticed a difference in gender. Hers is the first and only model that I am aware of in which gender differences have been reported, which made it a natural model for us to use," explained Kebebew.

Back to Cancer

"Later on, we looked back in the literature and found there are correlative studies to indicate that abnormal expression and somatic mutations of the *THRB* gene are found in human cancers," said Cheng, describing the evolution of

(*Photo: R. Baer*)

Sheue-Yann Cheng, Ph.D.

There and Back Again

(*Photo:* S. O'Neal)

Celine Guigon, Ph.D.

Celine Guigon, Ph.D., is Dr. Sheue-Yann Cheng's most senior Research Fellow, whose five-year tenure in the Gene Regulation Section has almost ended. She will be returning to France to begin independent research on the role of estrogen receptors in ovarian cancer at the University of Paris, Diderot.

"During my doctoral work, I was interested in female fertility. So I studied ovarian physiology and pathology. And I was curious about the role of estrogens in ovarian cancer. First, however, I felt it would be important to gain some broader experience," said Guigon. Guigon found Cheng's advertisement for a research fellow on the NIH Web site and realized that the opportunity was a perfect match with her interests.

Guigon created mutant mice that combined the PV mutation of *THRB* with a *PTEN* mutation, to study their synergistic action in the development of multiple cancer types."Meanwhile, I was involved in about five different projects," said Guigon. With nine coauthored publications to date from the Cheng laboratory, Guigon has no shortage of evidence to back up her claims to productivity. She is also the proud mother of a two-year-old son.

Returning to France, Guigon will be able to establish an independent research agenda without having to build her own laboratory. "Now, I want to use the background of my doctoral and postdoctoral work to develop a new direction for my research. I applied for several grants this year and was funded to begin some studies of my own."

> You never know where life—or science—will take you.

her entry into the field of cancer research after generating the first PV mutant mice. "We realized that the thyroid hormone receptor β could be a tumor suppressor."

Guigon is testing that hypothesis by looking at the propensity of PV mutant mice to develop mammary cancers. "We knew that *PTEN*-deficient female mice are a good model for mammary gland tumors," said Guigon. They also found that the homozygous PV mutation alone could enhance abnormal growth (hyperplasia) of mammary glands, although mammary gland tumors were only found in about eight percent of mice homozygous for the PV mutation. Hypothesizing that the homozygous PV mice were dying before they could develop mammary tumors, Guigon added a single *PTEN* mutation to heterozygous PV mice, which normally do not develop tumors or die prematurely.

"With the two mutations together, the frequency of mammary gland tumors is much greater than with the *PTEN* mutation alone," said Guigon. Their evidence to date suggests that the two mutations interact through critical intracellular signaling pathways. They are currently working to elucidate those mechanisms and further establish the role of the thyroid hormone receptor β in tumor suppression.

Serendipity in Science

"I came to the NIH because I was following my husband, who was recruited from Chicago to work for the Nuclear Regulatory Commission in Washington, DC," recalled Cheng. "But I was very excited by the opportunity." Cheng transitioned from working on estrogen receptors at the University of Chicago to working on thyroid hormones at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

"I became a Principal Investigator at the NCI in 1979," said Cheng, "But at the time, I worked more on hormone actions than cancer research. I thought I would be studying thyroid hormones, and now I am studying cancer.

"You never know where life—or science—will take you," added Cheng. "But you've got to embrace it. In science, you can't proscribe what people do; they need to have a passion for it. Research is not that easy—it is not nine-to-five work. The NIH is a great place for people who have a passion for science, who are interested in what they are doing, who love what they are doing. A lot of times, I just can't wait to come to work. Every morning, I wonder what I'm going to hear from my fellows or what I'm going to discover."

To learn more about Dr. Cheng's research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=sycheng.